

Amendment Dated September 24, 2007  
Reply to Office Action of March 23, 2007

**Amendments to the Specification:**

The paragraph numbers and page numbers of the published application (US 2004/013730) have been referred to below to indicate the location of the amendments to the specification.

Please replace paragraph [0001], at page 1, with the following rewritten paragraph:

This application is a divisional application of United States Patent Application Serial No. 09/673,896 (which issued on February 24, 2004 as U.S. Patent No. 6,696,062), filed December 18, 2000, which is the National Stage Application of International Application No. PCT/EP99/02766, filed April 20, 1999 which was published under PCT article 21(2) in English, which claims the benefit of priority of Great Britain Patent Application Serial No. 9808866.9, filed April 24, 1998. The disclosures of these applications are herein incorporated by reference in their entirety.

[0134] 37  
Please replace paragraph [0151], at page 12, with the following rewritten paragraph: KD  
5/12/08

A preferred oil-in-water emulsion comprises a metabolisable oil, such as squalene, alpha tocopherol and Tween TWEEN® 80 ((80)-sorbitan mono-9-octadecenoate poly(oxy-1,2-ethanediyl); Uniquema/ICI). In a particularly preferred aspect the antigens in the vaccine composition according to the invention are combined with QS21 and 3D-MPL in such an emulsion. Additionally the oil-in-water emulsion may contain span 85 and/or lecithin and/or tricaprylin.

[0134] 37  
Please replace the paragraph [0152], on page 12, with the following rewritten paragraph: KD  
5/12/08

Typically for human administration QS21 and 3D-MPL will be present in a vaccine in the range of 1 $\mu$ g-200 $\mu$ g, such as 10 $\mu$ g-100 $\mu$ g, preferably 10 $\mu$ g-50 $\mu$ g per dose. Typically the oil in water will comprise from 2 to 10% squalene, from 2 to 10% alpha tocopherol and from 0.3 to 3% TWEEN® 80 ((80)-sorbitan mono-9-octadecenoate poly(oxy-1,2-ethanediyl); Uniquema/ICI). Preferably the ratio of squalene:alpha tocopherol is equal to or less than 1, as this provides a more stable emulsion. Span 85 may also be present at a level of 1%. In some cases it may be advantageous that the vaccines of the present invention will further contain a stabiliser.

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[Q36]

38

Please replace the paragraph [0153], on page 12, with the following rewritten paragraph:

KD

5/12/08

Non-toxic oil-in-water emulsions preferably contain a non-toxic oil, e.g. squalane or squalene, an emulsifier, e.g. Tween, TWEEN® 80 ((80)-sorbitan mono-9-octadecenoate poly(oxy-1,2-ethanediyl); Uniquema/ICI), in an aqueous carrier. The aqueous carrier may be, for example, phosphate buffered saline.

[0170]

53

Please replace the paragraph [0213], on page 16, with the following rewritten paragraph:

KD

5/12/08

Briefly, 15 $\mu$ l ( $>10^8$  cells/lane) of each sample treated with sample buffer (10 min at 95°C) are put into a SDS-PAGE gradient gel (Tris-glycine 4-20%, Novex, code no. EC60252). Electrophoretic migration occurs at 125 volts for 90 min. Afterwards, proteins are transferred to a nitrocellulose sheet (0.45 $\mu$ m, Bio-rad code no. 162-0114) at 100 volts for 1 hour using a Bio-rad Trans-blot system (code no. 170-3930). The filter was blocked with PBS-0.05% Tween TWEEN® 20 overnight at room temperature, before incubation with the mice sera containing the anti-BASB006 antibodies. These sera are diluted 100 times in PBS-0.05% Tween TWEEN® 20 and incubated on the nitrocellulose sheet for two hours at room temperature with gentle shaking, using a mini-blotter system (Miniprotean, Bio-rad code no. 170-4017). After three repeated washing steps in PBS-0.05% Tween TWEEN® 20 ((20)-sorbitan mono-9-octadecenoate poly(oxy-1,2-ethanediyl); Uniquema/ICI) for 5 min., the nitrocellulose sheet is incubated at room temperature for 1 hour under gentle shaking with the appropriate conjugate (biotinylated anti-mouse Ig antibodies from sheep, Amersham code no. RPN1001) diluted at 1/500 in the same washing buffer. The membrane is washed three times as previously, and incubated for 30 min. with agitation using the streptavidin-peroxidase complex (Amersham code no. 1051) diluted at 1/1000 in the washing buffer. After the last three repeated washing steps, the revelation occurs during the 20 min. incubation time in a 50 ml solution containing 30 mg 4-chloro-1-naphthol (Sigma), 10 ml methanol, 40 ml PBS, and 30 $\mu$ l of H<sub>2</sub>O<sub>2</sub>. The staining is stopped while washing the membrane several times in distilled water.

[0179] 53  
Please replace the paragraph [0216], on page 17, with the following rewritten paragraph:  
KD  
5/12/08

5 $\mu$ g of partially purified BASB006 protein mixed with two other *Neisseria meningitidis* serogroup B proteins are put into a SDS-PAGE gradient gel (4-20%, Novex, code no. EC60252) for electrophoretic migration. Proteins are transferred to nitrocellulose sheet (0.45 $\mu$ m, Bio-rad code no. 162-0114) at 100 volts for 1 hour using a Bio-rad Trans-blot system (code no. 170-3930). Afterwards, the filter is blocked with PBS-0.05%~~Tween~~ TWEEN® 20 ((20)-sorbitan mono-9-octadecenoate poly(oxy-1,2-ethanediyl); Uniquema/ICI) overnight at room temperature, before incubation with the human sera. These sera are diluted 100 times in PBS-0.05%~~Tween~~ TWEEN® 20, and incubated on the nitrocellulose sheet for two hours at room temperature with gentle shaking, using a mini-blotter system (Miniprotean, Bio-rad code no. 170-4017). After three repeated washing steps in PBS-0.05%~~Tween~~ TWEEN® 20 ((20)-sorbitan mono-9-octadecenoate poly(oxy-1,2-ethanediyl); Uniquema/ICI) for 5 min., the nitrocellulose sheet is incubated at room temperature for 1 hour under gentle shaking with the appropriate conjugate (biotinylated anti-human Ig antibodies, from sheep, Amersham code no. RPN1003) diluted at 1/500 in the same washing buffer. The membrane is washed three times as previously, and incubated for 30 min. with agitation using the streptavidin-peroxidase complex (Amersham code no. 1051) diluted at 1/1000 in the washing buffer. After the last three repeated washing steps, the revelation occurs during the 20 min. incubation time in a 50 ml solution containing 30 mg 4-chloro-1-naphthol (Sigma), 10 ml methanol, 40 ml of ultra-pure water, and 30 $\mu$ l of H<sub>2</sub>O<sub>2</sub>. The staining is stopped while washing the membrane several times in distilled water.